Supporting Information

Nanocalorimetric platform for accurate thermochemical studies in microliter volumes

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1. Numerical simulation of characteristic thermal time constants

Numerical simulations are based on a two-dimensional model which is shown in Fig. 1S(A). Modelling of the heat transfer phenomena in the isothermal holder was carried out using Comsol Multiphysics. The simulations take into account the physical properties (mass density ρ, thermal conductivity k and heat capacity cp) of the different parts and materials of the isothermal holder, in particular, the silicon sensor chip membrane, the polystyrene reservoir membrane, the polycarbonate reservoir, the aluminum parts, the PMMA top plate, as well as partially water-filled reservoir (sample), and air gaps. Additionally, the model includes also the ceramic chip frame, the printed circuit board and the metal pins of the sensor chip contacts. Boundary conditions are determined by: (i) the initial temperature of the platform at 20°C (ambient temperature, TAMB); (ii) the flat constant heater source QHEATER, for heating the isothermal holder, (iii) a local heat source QSAMPLE for heat generated by the sample, (iv) the natural convection at the external surfaces of the holder which is expressed by a convective heat transfer coefficient hCONV, estimated to 10 W/m²K for free convection of air.

The first transient study aimed both at simulating the thermal gradient observed when heating up the isothermal holder to 25 °C, and at quantifying the time scale for reaching the setpoint temperature. For this study, the only heat source applied is the flat constant heat source in the upper part of the holder (QHEATER at 20 W), which is switched on at t = 0. The results are summarized in Fig. 1S(B) and 1S(C). Fig. 1S(B) shows the temperature distribution at two different time points showing the evolution of the thermal gradients in the platform over time. Shortly after switching on the heat source (t = 1 min) the temperature of the holder is still very close to the initial temperature (20°C). However, a significant thermal gradient of up to about 60 mK arises in the sensing area (visible in the magnification inset of the sensing area at t = 1 min). This gradient in the sensing area decreases as the holder approaches the target temperature, as shown in the same figure (for t = 120 min). The corresponding curves for the average temperature in the sensing area (taken at the position of the hot junctions of the thermopile) THOT and for the thermal gradient ΔTHOT-COLD between hot and cold junctions as a function of time are shown in Fig. 1S(C). Assuming an exponential increase of the temperature THOT from 20 °C to the final temperature TF of 25 °C, the temperature profile may be described as:

\[ T_{\text{THOT}} = T_{\text{AMB}} + (T_{\text{F}} - T_{\text{AMB}}) \cdot (1 - e^{-t/t'}) \]

where t' is the time constant of the system. The time constant t' may thus be defined as the time t = t' at which the temperature TTHOT is equal to TAMB + 0.63(TF-TAMB). The estimated value for the system is 40 min which is reasonably short for common experiments. After 3 t' the system has approached the setpoint temperature and thermal gradients over the sensing area are negligible.

The characteristic time constant t'', which corresponds to the response time of the system upon heat that is generated by the sample located on the sensing membrane, was determined in a second transient study. Fast sensing response times improve the time resolution of dynamic measurements of heat-generating samples or reactions. This simulation was carried out by assuming the platform temperature to be stabilized at 25 °C and by applying a local heat source QSAMPLE of 40 μW at t = 0. Assuming an exponential increase of the temperature gradient ΔTHOT-COLD, the time constant t'' may be defined similarly to the previous case and it has been determined to be 8 s. The temperature distribution in the sensing area is shown in Fig. 1S(D) for t = 1 s and t' = 3 t'' = 24 s, while the corresponding time evolution of the temperature gradient ΔTHOT-COLD between hot and cold junctions is shown in Fig. 1S(E). The results of this second transient study demonstrate that the sensing response time is adequate for good time-resolved detection of most chemical/biochemical processes and that...
maximum thermal gradients of few tens of microkelvin are expected for tens of microwatt of heat power generated by the sample.

2. Experimental evaluation of platform stability and sensitivity

The temperature stability of the platform was evaluated at 25 °C, which is the temperature set for all subsequent experiments. Proportional-integral-derivative (PID) control parameters have been optimized to obtain a good compromise between fast response time and minimal oscillation around the setpoint temperature. The temperature $T_{PID}$ acquired by the PID sensor over time is shown in Fig. 2S(A). The setpoint temperature of 25 °C is reached after about 10-15 min, whereas maximum temperature stability of ±1 mK is achieved after 25 min. This value indicates an extremely good thermal stability of the platform. The accuracy of measurement was limited by the resolution of the temperature read-out (see the magnified inset in Fig. 2S(A)). The temperature stability of the platform is expressed by $\pm \sigma_T$, where $\sigma_T$ is the standard deviation of temperature in the time window of interest (in this case from 25 min to the end of the measurement). The difference observed between the computationally estimated time constant of the system $\tau'$ of 40 min and the experimental thermalization time of 25 min arises from assuming a constant heat source in the thermal transfer coefficient ($h_{CONV}$).
simulation, corresponding to the minimum heat power required to reach the target temperature. Instead, in the experimental condition, the PID controller temporarily applies a much higher heat power to the heater, and controls it dynamically to maintain the setpoint temperature. Simultaneously, the corresponding thermopile voltage $V_{TP}$, shown in Fig. 2S(B), was recorded. No sample reservoir was placed on the membrane in this case. Higher voltage signals are observed in the beginning of the thermalization phase, reflecting high thermal gradients generated while heating up the platform. The thermopile voltage stabilizes after the setpoint temperature is reached, once thermal gradients over the sensing area have decreased significantly. This behavior is confirmed by the simulations shown in Fig. 1S(B). For this specific test, the thermopile voltage $V_{TP}$ has an offset of about 7 $\mu$V and a baseline drift of about 0.3 nV/s V (for 20 min < t < 40 min). Thus, a baseline subtraction algorithm was developed, consisting in putting to zero the baseline signal for each voltage measurement, and it was subsequently applied to all measurements.

Taking $\sigma_V$ as the standard deviation of the voltage $V_{TP}$ after baseline correction, the voltage signal stability can be estimate to $\pm \sigma_V = 80$ nV. Fig. 2S(C) shows three curves of the voltage $V_{TP}$ over time when very low heat power signals $P_{R, INT}$ in the range of a few hundreds of nW were generated, by means of the integrated resistive heater $R_{INT}$ on the sensing membrane (110 nW, 250 nW and 440 nW, respectively). The heat is generated by increasing gradually the electrical current through the integrated resistive heater.

3. Experimental protocol for sequential chemical testing

The sequential experimental injection procedure consisted in prefilling the reservoir with 45 µl of 1-propanol in water solutions at different initial concentrations. The inlet tubing connecting the reservoir was filled with up to 15 µl of pure 1-propanol. After allowing a thermalization time of 20 min to reach the setpoint temperature of 25 °C, sequential injections of 5 µl of pure 1-propanol were implemented. A waiting period of 20 min was allowed before each injection to ensure thermalization of the platform.

![Fig. 2S](image-url) Experimental evaluation of the temperature stability and of the background signal at 25 °C. A) PID sensor temperature $T_{PID}$ of the isothermal holder over time, when activating the flat heater on the top of the platform at t=0. After about $t_{stab} = 25$ minutes of thermal stabilization, the temperature stability of the platform is ± 1 mK ($T = 25.000 \pm 0.001$ °C). B) Corresponding thermopile voltage $V_{TP}$ over time curve. After thermalization of the platform ($t > t_{stab}$), the voltage has a slight baseline drift at a rate of about 0.3 nV/s (see inset in fig. 3B). A baseline correction has been applied to all subsequent plots. After correction, the voltage signal stability is ±80 nV. C) $V_{TP}$ signal over time measured when increasing heat power $P_{R, INT}$ pulses (110 nW, 250 nW and 440 nW, respectively) are applied to the resistive heater $R_{INT}$ integrated in the silicon membrane (platform is stabilized at 25 °C).
A time of 10 minutes was applied between subsequent injections to allow both the thermopile voltage signal to return to its initial baseline and the 5 µl sample to thermalize at 25 °C before its injection. Experimental results obtained are like shown in Fig.3S: here three separate heat peaks are observed for each injection, and analogues calculations to the ones described in the paper for estimating the enthalpy of mixing may be applied to each injection separately. The multiple injection approach is more time-efficient and particularly interesting when testing the effects of increasing concentrations of reactants.

**Fig. 3S** Heat power signal generated by sequentially injecting and mixing three 5 µl samples of pure 1-propanol into an initial 45 µl of 1-propanol solution, at 25 °C. The initial concentration and the final concentration of 1-propanol in the water was 69% and 77%, respectively.